the tumour the dog was unsuccessfully inoculated from a vaginal growth. Another dog was successfully inoculated from the same growth at the same time.

3. A white wire-haired puppy was successfully inoculated in both flanks. The resulting tumours, which reached the size of $1\frac{3}{4}$ in. in diameter, had completely disappeared in three months. The dog was then unsuccessfully inoculated from a growth in the subcutaneous tissue. Two other dogs were successfully inoculated from the same growth at the same time. Five weeks later the dog was again unsuccessfully inoculated from a subcutaneous tumour. Another dog was successfully inoculated from the same tumour at the same time.

4. A black and white terrier puppy was successfully inoculated from a vaginal growth in both flanks. The resulting tumours reached the size of about 2 inches in diameter and then ulcerated, and completely disappeared in six months. The dog was then unsuccessfully inoculated from a subcutaneous tumour. Another dog was successfully inoculated from the same tumour at the same time.

Conclusions.—1. The tumours in question are infective round-celled sarcomata occurring in dogs.

2. The tumours can be transplanted from the genitals, where they naturally occur, to the subcutaneous tissue of other dogs.

3. The tumours can be transplanted from subcutaneous tissue to subcutaneous tissue through a series of dogs.

4. The tumours after reaching the maximum of growth may disappear spontaneously with or without ulceration.

5. The tumours may continue to increase, and may cause death by secondary deposits forming in the viscera.

6. If the tumour should disappear the animal is then immune to subsequent inoculation.

REPORT OF THE COMMISSION FOR THE INVESTIGATION OF FOOT-AND-MOUTH DISEASE AT THE INSTITUTE FOR INFECTIOUS DISEASES, BERLIN.

By Geh. Med-Rath Prof. Dr LOEFFLER and Prof. Dr FROSCH.¹

I.

BERLIN, 17th April 1897.

(1) *Investigations Regarding the Etiology of the Disease.*—Thanks to the telegraphic reports regarding fresh outbreaks of foot-and-mouth disease which reached the Institute for Infectious Diseases, a large quantity of fresh material was available for examination. For this purpose places were selected within easy distance, so that it was possible to return the same day and examine the materials obtained while they were still quite fresh. The best material for investigation appeared to be the contents of quite recently developed vesicles in the mouth and on the udder of affected animals, because from such places the contents of the vesicles could be obtained free from accidental impurities, whereas it is very difficult to obtain matter in an uncontaminated condition from the feet, even when the most careful disinfection of the skin has been carried out.

Since experience had shown that in vesicles which are several days old bacteria may have propagated into their interior, care was taken to use only such vesicles as were quite recent. Unfortunately, these are rarely to be

¹ "Berliner Thierärztlichen Wochenschrift."

found, and even in outbreaks affecting large numbers of animals only one or two animals furnished such vesicles. In collecting the lymph the surface of the vesicles was first treated with absolute alcohol, and the lymph was then taken in sterilised glass capillary tubes thrust into the interior of the vesicle. In this way the contents of vesicles from twelve animals were obtained and subsequently examined. The examination included a search by the aid of bacteriological methods (hanging-drop, staining, cultivation). The culture media used were ordinary bouillon, both acid and alkaline, peptone bouillon, grape-sugar bouillon, liquid and solid blood serum, milk, nutrient agar, and gelatine, and the cultures were made with exposure to air or in an atmosphere of hydrogen, sulphuretted hydrogen, or carbonic acid.

The results of all these different methods of investigation were absolutely identical. No bacteria of any sort could be detected by the examination of hanging-drop cultures. In the great majority of cases culture media inoculated from the contents of the vesicles and kept under observation for several weeks remained absolutely free from any bacterial growth, and in those instances in which bacteria did develop it was perfectly obvious that the colonies had resulted from bacteria which had accidentally gained access to the culture vessels. That in spite of this negative result of bacteriological examination the lymph did actually contain the cause of foot-and-mouth disease was evident from the fact that calves and yearlings which were inoculated with the same material on the mucous membrane of the upper or under lip always developed the disease in a typical way after two or three days; and that the vesicles thus produced were not the result of any poisonous substances in the materials employed was proved by the fact that the disease thus set up spread to healthy animals kept in the same place with the experimental ones. From the result of these experiments it may with certainty be concluded that any bacterium which grows on any of the nutrient media in common use is not the cause of foot-and-mouth disease. Nevertheless the Commission considered it necessary to submit to examination a particular species of bacterium which had been found by Drs Siegel and Bussenius in alleged fatal cases of foot-and-mouth disease in the human subject, and also in cases of the same disease in animals, and this appeared to be the more necessary because these authors had alleged that they had been able to produce typical foot-and-mouth disease in calves and pigs with pure culture of their bacillus.

The Commission placed themselves in communication with these gentlemen, and obtained from them several pure cultures of the bacillus in question. It proved to be an actively motile bacillus, and grew luxuriantly in ordinary bouillon, grape-sugar bouillon, peptone bouillon, nutrient agar, and gelatine. Isolated colonies of the bacillus, when growing in the last-mentioned substances, are easily distinguishable owing to a peculiar marking which they present when slightly magnified. The bacillus itself frequently shows a certain tinctorial peculiarity, inasmuch as it stains more deeply at the ends than in the middle, or in other cases its central parts appear stained, whereas the intermediate parts show little or no colour. This method of staining depends upon the action of certain salts in the nutrient media employed, and it is therefore not always observable. Inasmuch as, according to the views of its discoverers, the bacillus is mainly found in the blood of recently attacked animals, although frequently only in very small numbers, special attention was always directed to examination of the blood. In five cases blood was taken from the jugular vein of recently attacked animals by means of a sterile trocar, and collected in sterile Erlenmeyer flasks; blood was also taken from the heart of two calves which had been killed at the height of the disease immediately after development of vesicles. Large quantities of the blood were used to inoculate bouillon, nutrient agar, and nutrient bouillon, the flasks being then placed in the incubator. In the great majority of cases the flasks thus

inoculated remained permanently sterile, but in a few of them micrococci, and in some others bacilli, developed. Most of the latter belonged to the group of pseudo-diphtheria bacilli, and had not the most remote resemblance to the bacillus of Siegel and Bussenius. They were obviously accidental impurities which had been obtained from the skin of the animals in taking the blood.

These negative results of experiment are in contradiction with the positive assertion of the authors named, that they had succeeded in producing the typical disease with their bacillus; nevertheless the Commission considered it advisable to afford them an opportunity to demonstrate the experimental production of the disease with the bacillus. The Commission were mindful of the fact that experiments of this sort might easily lead to a false conclusion, since the disease might be conveyed to the inoculated animals by the attendants, or even by the members of the Commission themselves, who were almost daily brought into contact with animals suffering from foot-and-mouth disease. It was therefore decided to carry out the experiments in a stable, and on animals which had been purchased from a district known to be free from foot-and-mouth disease, as well as on two sucking calves bought at the slaughter-house, Berlin. The animals were attended to by a servant of the Institute who was forbidden to have any intercourse with the other servants.

Inasmuch as it was possible that the bacillus of Siegel and Bussenius might have lost some of its virulence from long cultivation in nutrient gelatine, at the desire of these two gentlemen an attempt was made to infect the two sucking calves by pouring about 50 ccm. of a two-days-old bouillon culture of the bacillus into the mouth of each animal. At the same time, however, two yearlings were inoculated by scarification on the upper and lower lips in the same manner as one proceeds in infecting animals with foot-and-mouth disease lymph. In the case of the yearlings the material employed was a fresh culture of agar, and a large quantity of the culture was also rubbed on the mouth of these two animals, so that an infection of the intestine was also made possible.

On the following day the sucking calves were already ill, with high fever and symptoms of intestinal affection. One of them was killed while very ill on the third day, and the other died in the course of the following night. In both of these animals the bacilli were found in the blood and spleen, and especially in the much swollen mesenteric glands, as well as in contents of the intestines. Their presence in these positions was demonstrable by microscopic and cultural examination. Neither of the animals had lesions in the mouth or on the feet, such as are characteristic of the disease in question. On the contrary, they were affected with a severe enteritis.

With a two-days-old bouillon culture started from the heart blood of the above calf in which death resulted naturally, three new animals were infected at the instigation of Drs Siegel and Bussenius, in order to induce a less acute form of the disease, and thus to give time for the production of the characteristic lesions. One of these was a sucking calf and it received 2 ccm. into the mouth. The second animal was a three-months-old calf, and the third a yearling, and each of these received 5 ccm. into the mouth. On the following day the sucking calf was already the subject of high fever and profuse diarrhoea, and it died on the fourth day. The *post-mortem* examination showed practically the same conditions as in the above-mentioned calves.

The three-months-old calf became ill on the second day, being also attacked with high fever and profuse diarrhoea. Subsequently, however, it recovered, though its temperature remained for a long time over 41° C. Both the inoculated yearlings, and also the one infected by feeding, sickened on the fourth day, with rather high fever and profuse diarrhoea. The fever lasted from two to four days, and as it declined the diarrhoea also abated. During

fourteen days' observation none of the animals showed any symptoms of foot-and-mouth disease.

From these experiments it follows that the bacillus of Seigel and Bussenius, although an interesting and remarkable pathogenic organism, capable of setting up severe intestinal disease, is not the cause of foot-and-mouth disease.

From the results of the investigations above described it may also be concluded that the bacteria found by other observers—Nosotti, Klein, Schotteluis, Kurth, Nissen, Starcovici, Furtuna, and Stutzer, in cases of foot-and-mouth disease, did not represent the causal agent of the infection.

There still remained for investigation the claims of certain observers who have described, not bacteria, but small protoplasmic structures with distinct amoeboid movements, as the cause of the disease. Claims of this sort have been put forward by Piana, Fiorenti, Behla, and Jurgens.

Numerous preparations made from lymph were searched for such structures. In the lymph there were constantly found the following morphological elements:—

1. Colourless lymph cells.
2. Granular cells.
3. Red blood corpuscles.
4. Delicate, pale, finely granular, round discs, without distinct nucleus, and varying in size, but on an average from a quarter to half the size of a red blood corpuscle; not seldom such structures contain one, two, or even three glancing granules.
5. Protoplasmic structures of irregular shape, sometimes more or less strongly refractile, devoid of a nucleus, and showing continual oscillating movements. These structures perhaps correspond with the things which have been put forward by the before-mentioned observers as the cause of foot-and-mouth disease, but the members of the Commission have not been able to convince themselves that they possess the power of independent amoeboid movement.

6. In every case there were found numerous markedly refractile granules of various sizes, from the most minute points up to the size of the discs described under number 4.

The structures described under 4 and 5 could be stained by different methods, and by double staining it could be made out that those described under number 4 are made up of two substances with different tinctorial properties. The appearance of these recalled in a remarkable way that of embryonic erythrocytes.

The members of the Commission were unable to confirm the view of the above-mentioned observers that the protoplasmic structures in question are found only in the contents of the vesicles of foot-and-mouth disease. These structures therefore cannot be regarded as specific for the disease in question. The multiplication of them in or on nutrient media inoculated with the contents of vesicles could never be made out. Even on culture media specially prepared for the purpose they could not be cultivated.

(2.) Apart from the discovery of the causal agent of foot-and-mouth disease, which the Commission had hitherto placed in the first line of their investigations, their enquiries were extended in a number of other directions, as follows:—

1. The transmission of the disease to the various species of animals.
2. The method of infection.
3. The material of infection.
4. The resistance of the virus.
5. The destruction of the causal agent.
6. The development of immunity after recovery from the disease.
7. The possibility of discovering a method of protective inoculation.

Transmission of the Disease to Different Species of Animals.—The only animals that we have been able to infect experimentally with certainty are cattle (2) or calves (13). Out of twenty-two attempts to infect pigs only eight were successful. There was no success in eight attempts with sheep, and only one out of the same number with goats. Attempts were also made to infect 30 rabbits, 14 guinea-pigs, 3 dogs, 4 cats, 5 rats, 10 house mice, 10 field mice, 6 hens, and 6 pigeons, but the attempts failed, whether they took the form of inoculation into the mucous membrane of the mouth or into the limbs, intraperitoneal injection, or feeding with fresh materials.

Mode of Infection.—According to experience, in adult cattle and calves infection follows when materials moistened with fresh slaver from diseased animals, such as towels, sponges, or wisps of straw, are used to rub the mouth; but even in this way infection does not always follow with certainty. For example, in one instance only nine out of seventeen animals which it was sought to infect in this way on each of three successive days in the same building contracted the disease. The disease was experimentally transmitted to two yearlings and 13 calves by rubbing a drop of lymph on the slightly scarified mucous membrane of the upper and lower lips. The vesicles developed not only at the places scarified but also in their neighbourhood, on the palate and tongue. In the case of the yearlings vesicles developed on the feet, but in the great majority of cases the disease did not attack the feet in the calves. It was observed that the vesicles formed on the feet one or two days after the disease had shown itself in the mouth. On the second or third day after experimental infection the temperature rose 1° or $1\frac{1}{2}^{\circ}$, and in the course of the next three days it fell to the normal. The formation of vesicles set in from the first to the third day, generally on the second day, after inoculation. In the case of ten oxen inoculated in the skin of the back or on the posterior aspect of the thigh, no local lesion was produced, and up to the fifth day after inoculation none of the animals appeared to have contracted the disease. Unfortunately this experiment was vitiated by the fact that three days after the inoculation in the skin the owner had also attempted to infect the animals, so as to get rid of the disease as quickly as possible. After that the animals developed the symptoms of foot-and-mouth disease, but it was impossible to determine whether this was the result of the first or the second attempt to infect them.

In the case of a calf one-tenth ccm. of lymph was injected under the skin of the shoulder, and on the second day afterwards the typical lesions had developed in the mouth. It is possible that in this case the injection had been made into a blood vessel, for it was observed that a drop of blood exuded when the needle of the syringe was withdrawn. In the case of another calf 10 ccm. of blood serum taken from an animal at the height of the disease, and mixed with a drop of lymph from a vesicle, was injected under the skin, but the calf did not develop any symptoms of the disease during the next fourteen days. The question whether infection can take place from the subcutaneous tissue or from the blood stream, therefore, requires further experiments for its elucidation. Further investigation is also necessary to settle the practically important question whether an animal can be infected through the unwounded skin of the claws.

In three of the pigs experimentally infected the disease was transmitted by feeding, and in the others by inoculation between the claws. Cutaneous inoculation on the back and at the snout had negative results.

Infective Material.—The contents of freshly developed vesicles have been found to be the most certainly effective material for infection. Serum of blood taken from the jugular vein of diseased animals during the period of vesicle formation, and subcutaneously injected in quantities of from 10 to 14 ccm.,

did not produce the disease in three calves. Experiments regarding the infectivity of the urine and fæces of diseased animals have not yet been carried out.

Period during which the Virus remains Active.—The contents of capillary tubes kept in an ice chest sufficed to infect a calf, and in another case a pig, after twelve days.

Acquired Immunity.—Some veterinary surgeons are of opinion that one attack of the disease confers an immunity which lasts for one, two, or even three years, though it is said that this immunity is not observed in all animals. Three calves which, twelve, seventeen, and twenty-two days respectively after the first inoculation, were re-inoculated with material known to be active, became ill with the typical elevation of temperature, but without developing any lesions in the mouth or on the feet. Only the calf inoculated after the twelve days' interval showed several small abortive vesicles at the seat of inoculation in the mouth. Not one of the animals (four yearlings and twelve calves) which has come through an attack of the disease has had a second attack, although attempts have repeatedly been made to re-infect them with fresh material.

On the 9th of March two calves received by subcutaneous injection from 10 to 15 ccm. of blood serum from an ox suffering from the disease in an acute form. Both of the calves were inoculated again after seventeen days with the fresh contents of vesicles from the mouth, and as a result both became diseased, with moderate elevation of temperature and the formation of typical large vesicles in the mouth. A third calf which, on the 17th March, had received by subcutaneous injection 10 ccm. of serum taken from a typically diseased calf three days after its inoculation, was re-inoculated nine days later with active material. It reacted on the next day with an elevation of temperature, but did not contract the disease.

II.

BERLIN, 14th August 1897.

Method of Infection.—Through numerous comparative experiments it has been ascertained that the most certain method of infection is the introduction into the blood stream of the virus found in the contents of vesicles. Success has also attended the introduction of the virus into the peritoneal cavity, injection into the muscles, and rubbing of it into the mucous membrane of the mouth after wounding by pricking. On the contrary, inoculations into and under the skin have proved uncertain. Both of these appear to be effective only when at the same time the virus is introduced into a blood vessel. From the moment that the temperature begins to rise till the development of the local symptoms of the disease, the virus circulates in the blood stream, but it disappears from the blood after the development of the local lesions. The disease could be conveyed to healthy animals with from 50 to 100 ccm. of blood taken from the jugular vein of an animal twenty to twenty-eight hours after its inoculation.

After the introduction of the virus into the blood stream, the local lesions develop in quite a regular order thus:—

After from twenty-four to forty-eight hours the vesicles appear in the mouth, and in milch cows on the udder. After about other twenty-four hours the vesicles appear on the feet, and usually on all four at the same time. Putting aside the method of infection, the quickness with which the disease appears, and the intensity of the local lesions, depend upon the quantity and the virulence of the lymph.

Keeping Power of the Lymph.—The virus present in the contents of vesicles was rendered inactive:—

1. By twenty-four hours drying at summer temperature (maximum 31°C . at mid-day).

2. By heating at 37°C . for twelve hours (the effect of a shorter exposure at the same temperature was not tried).

3. By heating for half an hour at 70°C .

4. Heating for an hour at 60° makes the virus inactive. In one case, however, in which a large quantity of lymph was heated for half an hour at 60° , and then injected into an animal, a typical attack of the disease was set up. This observation shows that at the temperature mentioned a number of the germs are able to withstand exposure for half an hour.

Lymph in capillary tubes kept in an ice chest was certainly infective after fourteen days. After three weeks it was sometimes inactive, but even after having been kept for eight or nine weeks in the ice chest many samples of lymph were found to be capable of setting up the disease when injected in large quantities.

Quantity of Lymph necessary for Infection.—Fresh lymph was accurately measured and afterwards diluted with tap water which had been boiled, and experiments made with this mixture showed that $\frac{1}{50,000}$ ccm. was certainly infective. With smaller quantities, from $\frac{1}{100,000}$ ccm. to $\frac{1}{200,000}$ ccm., failures occurred, and infection could not be obtained with quantities of from $\frac{1}{300,000}$ ccm. to $\frac{1}{1,000,000}$ ccm.

Acquired Immunity.—The investigations of the Commission were mainly directed to determining whether animals which have come through an attack of the disease have thereby acquired immunity against a later infection. Only if this question can be answered in the positive sense is there a hope of being able to successfully deal with the disease.

Attempts have repeatedly been made to confer an individual protection on susceptible animals, but hitherto all experiments in this direction have failed to yield practical results. Most veterinary authorities have declared that there is no hope of doing anything in this direction, since it is alleged that one attack of the natural disease does not leave behind it any immunity. In consequence of these opinions, the Commission would have thought it hopeless to go into the question of immunity in foot-and-mouth disease had they not observed that a number of their own experimental animals were undoubtedly immune after having come through an attack of the disease. Repeated re-inoculation of animals that had had an attack showed that in the great majority of cases, in calves and adult cattle, immunity is present from two to three weeks after the development of the disease. Regarding the duration of this immunity, nothing more can be said at present than that it certainly lasts for five months. However, this immunity is not acquired in every case. Occasional animals when re-inoculated within a month after having suffered from the disease were attacked a second time. After that, however, they were immune. This behaviour of cattle with respect to foot-and-mouth disease corresponds very closely with what is observed in the human subject in regard to measles and small-pox, which are the infectious diseases that with the greatest certainty confer immunity against a second attack. But even in the case of these diseases occasional individuals may suffer from two or even three attacks.

Just as there are animals belonging to the bovine species which are highly susceptible to foot-and-mouth disease, there are others which appear to have a so-called natural immunity.

The Commission instituted a series of experiments, having for their object to discover some practicable method of conferring immunity :—

1. *Experiments with Fresh Lymph.*—Animals which had received into their veins active lymph so far diluted that it was no longer capable of infecting were found to have acquired no immunity. The same was found to be the case

with animals that had been inoculated cutaneously or subcutaneously with undiluted lymph, but had not thereby become infected.

2. *Experiments with Heated Lymph.*—A number of the animals which had been inoculated with lymph rendered inactive by heating showed a certain degree of immunity, especially those which had received large quantities of the lymph. The result, however, was not at all satisfactory, for when the whole of the animals were tested the number that contracted the disease was greater than the number found to be immune.

3. *Inoculation with a Mixture of Vaccine Lymph and Lymph from Foot-and-mouth Disease Vesicles.*—As has already been mentioned, animals which are cutaneously inoculated without contracting the disease do not thereby acquire any immunity. In order to bring about, if possible, a more active localised development of the causal agent of foot-and-mouth disease, experiments were made with mixtures of vaccine lymph and foot-and-mouth disease lymph. Some of the animals in which after inoculation with the mixture only a vaccine lesion developed at the point of inoculation were found when tested three weeks later to be immune against foot-and-mouth disease. On the other hand, inoculation with vaccine lymph alone was found to confer no immunity against foot-and-mouth disease; and, conversely, animals which had come through an attack of foot-and-mouth disease had no immunity against the vaccine lymph. It must therefore be concluded that when the mixed lymphs are inoculated a local multiplication of the causal agent of foot-and-mouth disease does actually take place, and confers immunity.

The experiments with mixed lymph were not pursued further, for it was found that when any bleeding occurred at the point of inoculation the animals were attacked with general foot-and-mouth disease. By means of a hammer heated in boiling water, blisters were raised on the inner surface of the ear, and when foot-and-mouth disease lymph was introduced into these without wounding the base of the blisters the animals did not become affected, and did not acquire any immunity, but if in performing the operation the base of the blister was wounded, so as to cause hæmorrhage, a typical attack of foot-and-mouth disease was the result.

4. *Experiments with the Blood of Immune Animals.*—In order to see whether a passive immunity could be conferred on calves by injections of blood from animals which had come through an attack of the disease and subsequently been repeatedly inoculated without result, a series of animals were treated with such "immune blood" in graduated quantities of from 10 to 150 ccm. The animals were then inoculated from twenty-four to seventy-two hours afterwards with the fresh contents of vesicles, with the result that they all contracted the disease in quite a typical form.

5. *Experiments with Mixed Lymph and Immune Blood.*—Experiments were now made to ascertain whether the blood of immune animals when directly mixed with active lymph is capable of robbing the latter of its infective power. Lymph and defibrinated immune blood were mixed in definite proportions, and a series of animals were injected with the mixture. In no case did the injection set up an attack of foot-and-mouth disease, whereas a mixture of lymph and blood from healthy non-immune animals, when used in the same proportions, was found to be without exception infective.

It thus appears that the blood of animals which have come through an attack of the disease prevents an undoubtedly active lymph from exerting its pathogenic properties in the body of a susceptible animal, although the blood of a non-immune animal has no such effect. Hence, during an attack of the disease there are formed in the body of the animal substances which have a specific action on the causal agent of the disease—an observation which is quite analogous to what is known to occur in many other infectious diseases.

The injection of a mixture of lymph and immune blood does not make the

animal obviously ill, whether it be injected under the skin or into the blood stream. In some of the animals there was a distinct rise of temperature, up to 40° C. or more, but in others there was no febrile reaction.

It was now of great interest to determine whether the animals had also acquired immunity through inoculation with the mixture of immune blood and lymph. Inasmuch as in natural cases of the disease the immunity appears to set in about three weeks after the attack, the animals treated with the mixture of lymph and blood were isolated as carefully as possible during this period, and then put into byres which were being constantly contaminated by animals recently attacked with foot-and-mouth disease. No disinfection of these byres was practised. After some days the animals were tested by the intravenous injection of active lymph. The great majority of the animals withstood this test, although in similar circumstances untreated animals always contracted the disease. The animals were kept under observation for five weeks, and during that time none of them were attacked.

The experiments of the Commission have thus shown that it is possible to confer an artificial immunity against foot-and-mouth disease on animals. The prospect of being able to introduce a practicable method of protective inoculation has thus been opened up.

III.

BERLIN, 8th January 1898.

In the preceding report the Commission have shown that it is possible to render healthy animals immune against foot-and-mouth disease. This result was obtainable in several ways, but the best results were obtained by using a mixture of lymph and blood of animals which had themselves acquired immunity in consequence of an attack of the disease. The Commission have therefore endeavoured to develop this method so as to make it available on a large scale as a means of combating the disease. A most important point to determine was the quantity of blood necessary to be added to the active lymph, so that the animals should not be infected by the mixture. With this object a series of inoculations were carried out on calves and pigs, the quantity of lymph being graduated while the quantity of immune blood remained constant in one of the series of experiments, and the quantity of immune blood being varied while the quantity of lymph remained constant in the other series.

These experiments showed that in order to confer immunity on calves the quantity of lymph used must not be too small. The quantity of fresh lymph necessary to immunise a calf was found to be $\frac{1}{40}$ to $\frac{1}{50}$ ccm. The quantity of immune blood to be added to this quantity of lymph was varied within rather wide limits, from 1 to 50 ccm. being employed. Naturally, the smaller the quantity found to be sufficient it would be the more convenient in practice. A series of experiments showed that 1 ccm. of immune blood sufficed to render innocuous the quantity of lymph previously ascertained to be necessary to confer protection. The lymph with which these results were obtained was taken from calves which had been inoculated in continuous series in the Institute. The results were so favourable that by means of this method ($\frac{1}{30}$ ccm. of fresh lymph and 1 ccm. of immune blood), the exact moment at which immunity sets in could be determined. It was found to be about three weeks.

On the ground of the absolutely satisfactory result of these laboratory experiments, it appeared to be justifiable to proceed to test the method in the conditions of everyday practice. An opportunity to do this presented itself in the neighbourhood of Greifswald, where, on two farms, Rappenhagen and

Boltenhagen, belonging to Mrs Becker, the disease broke out in the month of September. The disease had been introduced to Rappenhagen by some newly bought oxen, and it there attacked the draught oxen. It was then carried to Boltenhagen, and there also it attacked the lot of working oxen. From these it spread to the cow byre, in which there were 153 cows, a number of them being heavy in calf, and in the month of September most of these were attacked. At Rappenhagen there was a special shed containing fifty-four three year-old bulls, and at the same place there were at pasture other twenty bulls of from one-and-a-half to two years old. At Boltenhagen, on the other hand, there were two lots of young cattle at pasture, the numbers being thirty-four and thirty-two respectively. At the latter place there were also thirty-eight calves of various ages, some of them being in the cow byres, and the remainder in other sheds. An unusually large stock of cattle were thus threatened with the disease on both farms, and the Commission therefore had an extraordinarily favourable opportunity to test their method of protective inoculation. Abundance of fresh lymph was obtainable from the numerous animals acutely affected with the disease.

The necessary immune blood was easily obtainable from the immune animals kept in the sheds at Berlin. Through a mistake the blood was taken not from the most strongly immunised and repeatedly reinoculated animals, but from an animal which had been immunised by heated lymph and afterwards tested once as to its immunity with virulent lymph. The inoculation of the bulls and young cattle at the two farms was attended with great difficulty; the animals had first to be driven into a corner, and then each had to be lassoed over the horns. The young animals could then without difficulty be injected into the jugular vein, but each of the seventy-four bulls had to be cast and tied, as otherwise the inoculation was attended with danger to the lives of those performing the operation. Two very bad-tempered bulls had to be left uninoculated. All the operations were carried out in a perfectly satisfactory manner, and the results were as follows. The whole of the fifty-four three-year-old bulls were attacked with the disease from the sixth to the tenth day after the inoculation, and the twenty younger bulls after about twelve days. The attacks, however, were so slight that the animals could be left at pasture; only six of the older animals had to be brought into the house on account of vesicles on the feet. The animals continued to eat very well, but went somewhat stiffly on hard ground, and a few of them frothed at the mouth. In the lot of thirty-four young cattle about five were observed to froth at the mouth, and about the same number in the other lot. A number of them went stiffly for some days, but these also had no difficulty in eating, and retained a good appetite. Out of eleven calves which were inoculated in the infected cow-byre, six were removed into another shed immediately after the inoculation. None of these afterwards contracted the disease, even when infected calves were placed along with them. Of the five inoculated calves which were left in the cow-byre, two contracted the disease and three did not, whereas out of ten uninoculated calves left in the cow-byre, all except two were attacked with the disease. An accurate examination of the animals at pasture could, unfortunately, not be carried out, as there were not sufficient attendants to catch and examine every single animal. The owner was very well pleased with the mild and rapid course of the disease among the inoculated animals.

The most remarkable point in connection with these experiments was the fact that a whole series of animals were attacked, though only slightly, from the tenth to the twelfth day after the protective inoculation. The question therefore arises whether these attacks were determined by the protective inoculation itself, or were the result of natural infection of the animals not

yet rendered immune. Inasmuch as no symptoms of the disease were exhibited by the six calves from three to four months old which had been isolated in a separate place immediately after the injection of the mixture of lymph and immune blood, it does not appear probable that the attacks should be laid to the charge of the inoculation itself. The possibility was certainly not excluded that the infection had been carried by the numerous attendants who had to be employed to catch the animals for inoculation in the fields, and who also had to do with diseased animals in the infected byres, although attempts had been made to disinfect their hands, clothes, and boots with sublimate solution. Even although only a few of the animals had been infected by the attendants, it was, of course, possible that these few might have infected the others; and inasmuch as the period of incubation in natural cases of infection is usually from two to six days, the animals which were attacked at the end of the second week might have been infected from others that had contracted the disease spontaneously. It was, unfortunately, impossible to submit the animals in the pastures to a daily examination, and we had to rely on the statements of the attendant, who may easily have overlooked slight cases of the disease. Only in the case of one of the lots of young cattle, numbering thirty-four head, were the animals individually inspected. For this purpose they were brought into the sheds about three weeks after the inoculation. Two of these animals had not been inoculated, and among the whole lot twenty-two, including the two not inoculated, were found to show slight scars on the jaws, while four had erosions in process of healing, and eight showed no lesions. These facts also speak against the view that the inoculation had infected the animals.

However, the fact that the twenty young bulls were almost simultaneously attacked about twelve days after the inoculation still leaves it possible that these animals had been infected by the inoculation, and this supposition was supported by the result of an experiment which had been begun in Berlin shortly before the inoculations were practised at the above-mentioned farms. In order, if possible, to confer a certain immunity, seven calves, two or three weeks old, after they had been injected with a mixture of immune blood and lymph, were inoculated a second time with the same mixture composed of $\frac{1}{10}$ ccm. lymph and 1 ccm. immune blood. To our great surprise the whole of these animals were attacked towards the end of the second week after the inoculation. In each of them there developed slight, but at the same time unmistakable, lesions of foot-and-mouth disease in the mouth, though certainly not on the feet. This experience was in contrast with everything that we had observed before. The fact that these at one time immunised animals contracted the disease, though only in a mild form, could only be ascribed to the insufficient activity of the immune blood, or to an abnormally high virulence of the lymph employed. The immune blood was the same as had been used for the first immunisation, and, consequently, the cause of the disease must have been the high virulence of the lymph employed for the second protective inoculation; and as a matter of fact the lymph used for this second inoculation came from a severe outbreak of the disease in Posen. It was used for the immunisation experiment twenty-four hours after it was obtained. Since the inoculations at Rappenhagen and Boltenhagen were also made with fresh lymph from a severe type of the disease, it is therefore possible that the infection of the twenty young bulls was determined by this lymph. The lymph which we had used for immunising the various lots came from different animals, and it is very possible that the lymph employed to inoculate these bulls may have been specially virulent.

It thus appeared that in experiments regarding immunisation by means of a mixture of blood and lymph, the virulence of the lymph as well as its quantity is an essential factor. It had therefore to be ascertained by new

experiments whether and by what quantity of immune blood this highly virulent lymph could be made safe for the animals to be inoculated. The experiments carried out with this object showed that $\frac{1}{40}$ ccm. of this highly virulent lymph could, by admixture with 10 ccm. of defibrinated blood from a well-immunised animal, be so influenced that the animals therewith inoculated either did not develop any disease or showed only some slight alterations in the mouth, unaccompanied by any injurious effect on the general health. These alterations, which were observed in a number of animals from ten to fourteen days after the protective inoculation, took the form of flat, circular, or streak-like exfoliation of the epithelium, occurring at the places where the characteristic vesicles are formed. These erosions are usually associated with accumulation of a blackish or brownish pigment.

Without careful daily examination of the animals these alterations, occurring so long after inoculation, would doubtless have been overlooked, inasmuch as the animals did not exhibit any other symptoms of disease. We were also for a long time in doubt whether they ought to be regarded as belonging to foot-and-mouth disease at all. However, since they were exclusively found in animals which had been injected with the mixture of immune blood and lymph, we arrived at the conviction that they could be nothing else than quite mild abortive products of the virus which had been weakened by the immune blood. In pigs which were treated by the same mixture of immune blood and lymph we have never observed such local alterations.

Through some experiments we have been able to convince ourselves that it is not necessary to mix the lymph and immune blood intimately with one another before injecting the mixture. The lymph was also rendered innocuous when it was separately injected into the jugular vein, followed by the injection of immune blood into the corresponding vein of the opposite side.

With regard to the immunising effect of the injection of lymph and immunised blood, we have ascertained that in the case of pigs, which are but slightly susceptible to the disease, about 95 per cent., and in the case of highly susceptible calves about 75 per cent., three weeks after the protective inoculation will bear, without afterwards exhibiting any symptoms of disease, the intravenous injection of 100 times the quantity of lymph which will suffice to infect an unprotected animal. This result must be regarded as very satisfactory, in view of the fact that not every animal that has come through an attack of the disease is thereby rendered immune. Should the infection be introduced into a lot of animals that have been submitted to protective inoculation, at the most a quarter of them will become infected. The danger of such introduction of the disease, however, will be relatively slight, seeing that the virus so introduced will find only a small number of susceptible individuals. The animals which have not been certainly immunised will derive protection from the fact that they are found in the company of animals that are protected, the conditions being similar to those which are observed with regard to vaccination against smallpox, where the individuals that have acquired little or no immunity are protected by the fact that the immense majority of persons have been certainly immunised. In our opinion, therefore, the practicability of the procedure is beyond question, although the ideal effect of conferring immunity on every animal in the herd has not been attained.

Before introducing the procedure into practice, it will be necessary to test it experimentally on a series of adult cattle. It is conceivable that the greater body-weight of adult animals may require the employment of a larger quantity of immune blood than is necessary for the immunisation of calves. It is also desirable to ascertain by accurate experiments whether the continued introduction of gradually increasing quantities of lymph into the blood stream of immunised animals will heighten the activity of the immune blood, so that

a smaller quantity than 10 ccm. of it might be employed for protective inoculation. Inasmuch as the virulence of the lymph is notoriously very variable in different outbreaks of the disease, experiments regarding the obtaining of lymph as constant in strength as possible require to be taken in hand.

Apart from the before-described results touching the practical question of immunisation, the Commission have still to report regarding others which are of no slight scientific interest, and which, as far as one can judge at present, have a far-reaching importance for the future investigation not only of foot-and-mouth disease but also of numerous other infectious diseases of man and animals.

In their endeavours to discover a practicable method of conferring immunity, the Commission have not confined themselves to experiments regarding the method of immunising by means of a mixture of immune blood and lymph, but have also taken into consideration other possible methods of conferring immunity, such as the introduction of blood from an animal at the acute stage of the disease, with or without the addition of immune blood, or the employment of lymph which has been freed from its corpuscular elements by filtration. It may immediately be said that the whole of these experiments have not yielded satisfactory results; on the other hand, the filtration experiments have yielded results which are of importance in the direction above indicated. In these experiments the lymph was diluted with thirty-nine parts of water, and to the mixture there was then added a large quantity of culture of an easily recognised species of bacterium, namely, the *bacillus fluorescens*. The liquid was then filtered two or three times through an unglazed porcelain filter. The object of adding the bacteria was to be able to ascertain by inoculating nutrient media with large quantities of the liquid whether the same was germ free. When no colonies of bacteria developed it could be concluded that all the bacterial elements present in the lymph had been kept back by the filter. The filtrate when tested in this way was always found to be free from bacteria. With this filtrate a series of calves were inoculated, quantities varying from $\frac{1}{10}$ to $\frac{1}{40}$ ccm. of the pure lymph being injected into the blood-stream in order to ascertain whether immunity could be conferred by any of the substances present in solution in the lymph.

The result of these injections was somewhat surprising. The animals which received the filtrate contracted the disease in the same time as control animals which had received a corresponding quantity of unfiltered lymph, and they all showed typical symptoms of the disease, with high fever and the development of vesicles in the mouth and on the feet. It appeared as if the activity of the lymph had not been affected by the filtration. In order to be quite certain regarding this point the experiments were several times repeated on a large number of calves and pigs. When fresh lymph was used the results were always the same, the animals treated with the filtered lymph always becoming affected in a quite typical way, just like the control animals treated with unfiltered lymph.

How was this striking fact to be explained? Two alternative explanations offered themselves—either the germ-free filtered lymph contained in solution some extraordinarily active poison, or the hitherto undiscoverable agent of the disease was so small that it could pass through the pores of a filter which will certainly keep back the smallest of the known bacteria. If the first explanation is the correct one, the soluble poison must be an amazingly active one. With a quantity of filtrate corresponding to $\frac{1}{30}$ ccm. of lymph, the disease could be set up in two days in calves of about 200 kg. In his experiments Brieger obtained bouillon cultures of the tetanus bacillus which were so strong that '0005 ccm. of the germ-free filtrate sufficed to kill a mouse; that is to say, 1 ccm. would kill 20,000 mice, and 1 litre would kill 20,000,000 of them. From this bouillon he obtained 1 gramme of a substance of which

·0000001 gr. killed a mouse; that is to say, 1 gramme of this substance would have sufficed to kill 10,000,000 mice. Judged by the lethal activity of the filtrate, 1 gramme of the substance ought to have killed 20,000,000 mice, but since it only killed 10,000,000, 1 gramme of the poison had been lost in its preparation. Accordingly, at the most, the poison must have formed $\frac{1}{500}$, or 2 per cent. of the bouillon.

If we now assume that in the lymph of foot-and-mouth disease vesicles the active material constitutes $\frac{1}{500}$ of the lymph, the quantity of this material in $\frac{1}{30}$ cm. of the lymph must be $\frac{1}{15000}$ gr. This quantity sufficed to communicate the disease in a typical way to a calf of 200 kg.; hence, provided that the poison was uniformly distributed throughout the whole body of the animal, one part of the poison must have been active when distributed in $15,000 \times 200,000$, that is to say, 3,000,000,000 parts of the calf. Out of tetanus cultures Knorr was able to extract a poison whose activity was 1 : 1,000,000 for rabbits; 1 : 150,000,000 for white mice; and 1 : 1,000,000,000 for guinea-pigs. In respect of its activity the toxin of foot-and-mouth disease would, therefore, far exceed that of the strongest tetanus poison. In the foot-and-mouth disease toxin we, therefore, had an agent of most astonishing activity.

Subsequently we were able to communicate the disease in a quite typical way in two days to a pig of about 30 kg. body-weight, by inoculating it with $\frac{1}{30}$ ccm. of lymph obtained from a vesicle on the foot of a calf which had received some of the filtered lymph. The poison must thus have experienced a further dilution of $1 : 50 \times 500 \times 3000$ (750,000,000). It is impossible to believe in a poisonous effect with such a degree of dilution.

In these calculations, as in those relating to the strength of the tetanus poison, it has been assumed that the toxin becomes uniformly distributed throughout the whole body of the animal into which it is introduced. But it is not certain that such is the case. In the case of a man or animal affected with tetanus, or poisoned with the tetanus toxin, the latter can be detected without difficulty in the whole mass of blood and in all the organs, but one cannot do the same in animals affected with foot-and-mouth disease. Large quantities of the blood of such animals (50 to 100 ccm.) must be used in order to infect healthy animals. It therefore appears to be quite possible that the whole or the greater part of the toxic substance introduced into the blood-stream within a short time afterwards becomes deposited in those parts of the body at which the vesicles develop. If we suppose that, in the case of an animal which has contracted a typical attack of the disease after the injection of $\frac{1}{30}$ ccm. of filtered lymph, the contents of the whole of the vesicles on the feet and in the mouth amount to 5 ccm., then the original quantity of toxin in $\frac{1}{30}$ ccm. of lymph would now be found in 5 ccm. of lymph, since it has been diluted 150 times. But of this lymph $\frac{1}{30}$ ccm. when injected into the veins sufficed to infect a pig of 30 kg. body-weight in two days. Hence, $\frac{1}{7500}$ of lymph, or, calculated at the same rate as in the tetanus experiments, $\frac{1}{3750000}$ of the toxin, must have exerted its action in 30,000 grs. of the pig or in 2000 grs. of blood. Even according to this calculation, the hypothetical toxin of foot-and-mouth disease present in the lymph must have had most remarkable activity. The view, therefore, that the effects of the filtrate were due, not to any soluble substance in the lymph, but to some agent capable of multiplication, cannot be dismissed as improbable, but in that case the agent must be so small that it can pass through the pores of a filter which will certainly keep back even the smallest bacteria. The smallest of the hitherto described bacteria is the influenza bacillus discovered by Pfeiffer. It has a length of from .5 to 1 μ . If we now assume that the supposed agent of foot-and-mouth disease is not more than $\frac{1}{10}$ or $\frac{1}{5}$ as large as this, then, according to the calculations of Professor Abbe, it would not be

visible under the highest powers of our microscopes, even with the best modern immersion lens. This would furnish a very simple explanation of the futility of attempts to discover the germ in the lymph by means of the microscope.

IV.

BERLIN, 12th August 1898.

In the preceding report it was mentioned that the lymph taken from fresh vesicles, when diluted with water and then filtered so as to deprive it of all bacteria, still retains its infective power. Further investigations have established the correctness of this observation beyond any doubt. It has been possible to show that by the aid of such filtered lymph the disease can be transmitted with certainty from animal to animal in a whole series of experiments. In such a series the sixth animal contracted the disease just as promptly as the first.

In support of the view that the agent of infection is some corpuscular element, and not a substance in solution, it was on many occasions observed that diluted lymph which was repeatedly passed through a thick Kitasato filter would no longer infect susceptible animals, even when one used a quantity of it representing $\frac{1}{10}$ ccm. of the pure lymph. Apparently the agent of infection had been kept back in the narrow pores of the Kitasato filter. Before carrying out the experiments in which the disease was transmitted in succession to a series of six animals by means of filtered lymph, it had to be shown experimentally that it was possible to do this with unfiltered lymph. The first experiments made in this latter direction did not at once give satisfactory results. After the fourth, and frequently even after the third transference, the virulence was found to be very notably diminished. At first the animals took the disease in quite a mild form, and subsequently the disease did not appear to be transmitted at all, whether calves or pigs were used for the experiment. However, the difficulty presented by this loss of virulence was overcome by using cattle and pigs alternately in the attempts to carry on the disease. After that it was possible to undertake the experiments with the filtered lymph.

The results of these experiments were of considerable practical importance, inasmuch as they enabled the Commission to obtain a supply of lymph of nearly constant virulence, and thus made them independent of outbreaks in the country, from which they had formerly been obliged to provide themselves with fresh lymph at considerable difficulty and cost.

A means of determining with certainty the degree of virulence of the lymph, by ascertaining the fatal dose for small animals, was not arrived at by the Commission, notwithstanding numerous experiments on mice, young guinea-pigs, young rabbits, young cats, young calves, and various species of small birds. Geese, which according to the views of some observers are very susceptible, withstood the injection of large quantities of lymph (1 ccm.) without any other effect than a rise of temperature. That the strain of lymph used by the Commission had attained a high degree of virulence was shown by the fact that it was found to be possible to infect goats with it, although in the earlier experiments these animals had appeared to be but slightly susceptible (out of eight animals only one was affected). It may be observed that the disease spread spontaneously from two experimentally infected goats to two other healthy goats kept along with them. The virulence of the lymph was further attested by the fact that several dogs (fox-terriers) which were running about in the buildings in which the diseased animals were kept developed symptoms of foot-and-mouth disease.

The Commission also instituted experiments regarding the possibility of infecting animals by introducing the virus into the stomach without allowing

it to come into contact with the mucous membrane of the mouth or upper part of the alimentary tract. A number of animals had administered to them gelatine capsules containing fresh lymph, these being introduced so far into the gullet that the animals were obliged to swallow them. After two or three days all the animals thus infected contracted the disease in a typical way. It must therefore be concluded that natural infection from the alimentary tract ought to be regarded as having considerable importance.

Numerous experiments were instituted regarding the best method of preserving the lymph. These showed that the germ-free filtered lymph retained its activity up to four months when kept in the ice chest. The addition of phenol, thymol, or even 5 per cent. of carbolic acid, did not appear to have any special importance.

Lymph diluted with ten times its volume of water and then rendered germ-free by filtration was found to have retained its virulence unaffected after eleven weeks, notwithstanding the addition of 1 per cent. of carbolic acid.

Whereas previous experiments had shown that pure undiluted lymph enclosed in capillary tubes lost its activity in twelve hours at 37° C., the undiluted filtered lymph at the same temperature was certainly infective after twenty-four hours, though it was found to be non-infective after three days.

None of the attempts to cultivate the hypothetical agent of the disease yielded successful results.

Immunisation Procedures.—As already explained in the report of the 8th January, it appeared to be necessary to institute further experiments before employing the method of immunisation devised by the Commission in practice. Such experiments were carried out on a great number of adult animals. In these experiments care was taken that the serum to be added to the lymph was obtained from animals which were able to withstand large doses of lymph without developing any local symptoms of the disease. It was now ascertained that some of the animals protectively inoculated, especially some of the adult cattle, contracted the disease after the injection of lymph and serum, whether 1, 5, 10, 20, 50, or 100 ccm. of serum had been mixed with $\frac{1}{30}$ ccm. of lymph. Even when the quantity of lymph was reduced to $\frac{1}{100}$, or to $\frac{1}{200}$ ccm. occasional animals still developed the disease, generally during the second week, about the tenth or twelfth day. Obviously if such results as this had to be expected in practice, protective inoculation on a large scale could not be carried out. The possibility of such an occurrence must in every case be excluded.

Experiments were, therefore, made with the serum from various species of animals after these had been repeatedly injected with large quantities of lymph. It was thus ascertained that, apart from cattle and pigs, the serum of horses and goats can be used to furnish the active agent of immunisation. For this purpose, on the other hand, the serum of geese was found to be nearly useless. However, even when the serum was taken from animals which had previously been injected with very large doses of lymph, occasional cases of disease were observed in the animals protectively inoculated with a mixture of the serum and lymph. In subsequent experiments the mixture of serum and lymph was not immediately injected, but allowed to stand for some time before it was used for protective inoculation, and it was observed that cases of the disease no longer occurred in consequence of the injection, provided the lymph and serum had been left for a sufficient time in contact.

It had then to be ascertained whether the animals thus treated had actually acquired immunity. Experiments to this end showed that almost without exception the animals, three weeks after the protective inoculation, could withstand a test inoculation with $\frac{1}{30}$ ccm. of the highly virulent lymph. Immunity set in even when the mixture of serum and lymph (10 to 20 ccm.

serum with $\frac{1}{50}$ ccm. lymph) had been prepared four weeks before it was injected. Immunisation was obtained quite as well in pigs as in cattle.

The activity of the sera of immune animals is subject to very considerable individual variations. Some animals furnish a serum so active that animals inoculated with a mixture of it and lymph will not contract the disease even when they are immediately placed in infected premises between animals suffering from the disease; indeed, they may withstand the intravenous injection of $\frac{1}{100}$ ccm. of lymph ten days after the protective inoculation.

In addition to the method of immunising by means of a mixture of serum and lymph, the Commission discovered still another method which, without doubt, might be employed to confer protection. As has already been mentioned, the filtered lymph loses its activity after several months. The lymph which has thus become inactive, when injected into healthy animals, protects them without setting up the slightest symptom of disease. All the animals thus treated with lymph which had been kept for six months were found to be immune when tested three weeks afterwards with $\frac{1}{50}$ ccm. of highly virulent lymph.

Opportunities to make extensive observations regarding the duration of the artificial immunity did not present themselves. Some animals (adult cattle and calves) which were set aside for this purpose were found to be quiet immune when tested three months after the protective inoculation.

The Commission made an interesting observation regarding the hereditary transmission of immunity. They had kept one of the first heifers which came through an attack of the disease, in order to subsequently test the duration of the animal's immunity. From time to time it received large quantities of lymph, and its serum was repeatedly used with success for immunising purposes. This animal gave birth to a calf on the 8th of May 1898, and three days afterwards $\frac{1}{100}$ ccm. of highly virulent lymph was administered intravenously to the calf. It did not become affected. Six days later it received the large dose of $\frac{1}{10}$ ccm. without developing the slightest reaction, and when afterwards placed in an infected byre it remained healthy.

In order to see whether any immunising substances are present in the milk of immune cows, the calf dropped by the experimental cow was taken away three days after its birth, and two recently purchased sucking calves were put to the cow. Fourteen days after this one of the calves was spontaneously attacked with foot-and-mouth disease, another experimental animal having developed the disease in the same premises. The second sucking calf contracted the disease in a typical way after the injection of $\frac{1}{200}$ ccm. of lymph; hence, nourishment for fourteen days with the milk of a certainly immune animal had not sufficed to protect either of the calves.